## **ORIGINAL ARTICLE**

# A new species of *Drosophila obscura* species group (Diptera: Drosophilidae) from China

Ji-Min Chen, Jian-Jun Gao\*

Laboratory for Conservation and Utilization of Bioresources, Yunnan University, Kunming 650091, China \*Corresponding author, E-mail: gaojj@ynu.edu.cn

**Abstract** A new species of the *Drosophila obscura* species group is described here, namely *Drosophila glabra* **sp. nov.** It was recently found from the Maoershan National Nature Reserve, Guangxi, China. The characteristics of the new species are based not only on morphological characters but also on DNA sequences of the mitochondrial *COII* (cytochrome *c* oxidase subunit II) gene.

**Key words** Genetic distance, morphology, Old World, Oriental Region, *Sophophora*.

#### 1 Introduction

The *Drosophila obscura* species group is one of the major lineages within the well-known sophophoran radiation recognized by Throckmorton (1975). Studies on the taxonomy, geography, chromosomal evolution, reproductive-isolation, protein polymorphism and phylogeny of this group have greatly promoted the early development of evolutionary genetics (Lakovaara & Saura, 1982). A majority of the currently known species of this group (44 in total) was recorded from the Holarctic temperate zone, with the remainder recorded from varied sites in South America (Lakovaara & Saura, 1982; Head & O'Grady, 2000), as well the Afrotropical (Séguy, 1938; Tsacas *et al.*, 1985) and Oriental Regions (Watabe *et al.*, 1996; Watabe & Sperlich, 1997; Gao *et al.*, 2003, 2009; Table 1). In this paper, we describe a new species of the *obscura* group found from our recent field survey in Guangxi, China. The definition of the new species is based on morphological characters and DNA sequences of the mitochondrial *COII* (cytochrome *c* oxidase subunit II) gene.

### 2 Materials and methods

The type specimens of the new species were collected using Toda's "retainer" traps (Toda, 1977) with banana bait in the Maoershan National Nature Reserve, Guangxi, Southern China in 2009. The specimens were then preserved in 70% alcohol and identified in laboratory. Most of the trapped specimens of the *obscura* group were identified as *D. hubeiensis* Sperlich & Watabe, 1997, with only two males recognized as of an unknown species in light of morphology. We then determined the DNA sequences of the mitochondrial *COII* (cytochrome *c* oxidase subunit II) gene for the two specimens using the same method as Gao *et al.* (2007), and calculated the pairwise K2P (Kimura two-parameter) distances between the two newly determined *COII* sequences and 28 previously-collected ones of the *obscura* group (O'Grady, 1999; Gao *et* 

Table 1. Distribution of the *Drosophila obscura* group from the Oriental Region.

Species subgroup	Species	Distribution
obscura		
	D. subobscura Collin, 1936	China (Xinjiang <sup>a</sup> )
	D. bifasciata Pomini, 1940	China (Xinjiang <sup>a</sup> , Heilongjiang <sup>b</sup> , Jilin <sup>b</sup> , Liaoning <sup>c</sup> ); India (Gulmag <sup>d</sup> , Pahalgam <sup>d</sup> )
	D. subsilvestris Hardy & Kaneshiro, 1968	China (Xinjiang <sup>a</sup> )
	D. tsukubaensis Takamori & Okada, 1983	China (Yunnan <sup>e</sup> , Shaanxi <sup>e</sup> , Guizhou <sup>e</sup> )
	D. limingi Gao & Watabe, 2003	China (Yunnan <sup>e</sup> , Guizhou <sup>e</sup> )
	D. dianensis Gao & Watabe, 2003	China (Shaanxi <sup>c</sup> , Yunnan <sup>e</sup> , Jiangxi <sup>c</sup> )
	D. epiobscura Parshad & Duggal, 1966	India (Pahalgam <sup>d</sup> )
sinobscura		
	D. sinobscura Watabe, 1996	China (Taiwan <sup>f</sup> )
	D. hubeiensis Sperlich & Watabe, 1997	China (Hubei <sup>g</sup> , Shaanxi <sup>c</sup> , Guizhou <sup>c</sup> , Jiangxi <sup>c</sup> , Fujian <sup>c</sup> , Guangxi <sup>c</sup> , Xizang <sup>c</sup> , Sichuan <sup>e</sup> , Yunnan <sup>e</sup> )
	D. luguensis Gao & Toda, 2003	China (Yunnan <sup>e</sup> , Xizang <sup>e</sup> )
affinis		
	D. helvetica Burla, 1948	India (Gulmag <sup>d</sup> , Pahalgam <sup>d</sup> )
un-subgrouped		
	D. alpina Burla, 1948	China (Xinjiang <sup>a</sup> )
	D. hypercephala Gao & Toda, 2009	Malaysia (Sabah <sup>h</sup> )
	D. hideakii Gao & Toda, 2009	Malaysia (Sabah <sup>h</sup> )
	D. quadrangula Gao & Toda, 2009	Malaysia (Sabah <sup>h</sup> )

a. Watabe *et al.* (1993); b. Toda (unpublished data); c. Gao (unpublished data); d. Parshad & Duggal (1966); e. Gao *et al.* (2003); f. Watabe *et al.* (1996); g. Watabe & Sperlich (1997); h. Gao *et al.* (2009).

Table 2. Pairwise K2P distances of the COII sequences in the Drosophila obscura species group.

	Species	GenBank accession number	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
1	glabra (No. 001867)	KP257569															
2	glabra (No. 001868)	KP257570	0.015														
3	affinis	M95140	0.083	0.087													
4	algonquin	M95144	0.072	0.079	0.009												
5	azteca	M95146	0.077	0.094	0.024	0.021											
6	athabasca	M95141	0.076	0.083	0.012	0.009	0.024										
7	tolteca	M95152	0.092	0.102	0.042	0.036	0.050	0.036									
8	narragansett	M95149	0.077	0.090	0.027	0.028	0.030	0.028	0.040								
9	helvetica	EF216252	0.084	0.100	0.059	0.052	0.062	0.052	0.065	0.062							
10	lowei	M95142	0.083	0.096	0.067	0.056	0.062	0.056	0.070	0.069	0.065						
11	miranda	M95148	0.090	0.092	0.063	0.056	0.061	0.061	0.085	0.072	0.076	0.062					
12	persimilis	M95143	0.094	0.100	0.065	0.055	0.060	0.058	0.083	0.070	0.070	0.047	0.032				
13	pseudoobscura	M95150	0.094	0.100	0.065	0.055	0.060	0.058	0.083	0.070	0.070	0.047	0.032	0.000			
14	limingi	EF216256	0.094	0.110	0.100	0.088	0.101	0.098	0.108	0.100	0.100	0.109	0.103	0.100	0.100		
15	tsukubaensis	EF216261	0.104	0.119	0.117	0.109	0.128	0.116	0.117	0.120	0.145	0.127	0.125	0.117	0.117	0.106	
16	tristis	EF216262	0.114	0.115	0.108	0.098	0.100	0.098	0.108	0.100	0.106	0.115	0.110	0.111	0.111	0.134	0.152
17	obscura	AF081356	0.111	0.107	0.122	0.114	0.122	0.116	0.120	0.120	0.118	0.130	0.115	0.111	0.111	0.130	0.147
18	ambigua	M95145	0.086	0.084	0.092	0.083	0.090	0.085	0.096	0.092	0.100	0.096	0.096	0.092	0.092	0.114	0.121
19	hubeiensis HB	EF216253	0.067	0.076	0.081	0.070	0.077	0.070	0.081	0.078	0.086	0.074	0.084	0.081	0.081	0.108	0.094
20	hubeiensis YN	EF216254	0.065	0.076	0.079	0.069	0.076	0.072	0.079	0.076	0.085	0.072	0.084	0.079	0.079	0.108	0.094
21	luguensis	EF216257	0.069	0.080	0.083	0.072	0.079	0.076	0.083	0.079	0.088	0.074	0.094	0.077	0.077	0.110	0.094
22	sinobscura	EF216259	0.067	0.078	0.083	0.072	0.079	0.076	0.083	0.079	0.088	0.072	0.088	0.083	0.083	0.112	0.098
23	subsilvestris	EF216260	0.087	0.090	0.083	0.072	0.074	0.072	0.090	0.083	0.083	0.068	0.094	0.086	0.086	0.112	0.127
24	dianensis	EF216251	0.079	0.092	0.083	0.072	0.070	0.072	0.083	0.079	0.074	0.070	0.086	0.085	0.085	0.102	0.123
25	bifasciata	M95147	0.089	0.107	0.107	0.096	0.107	0.103	0.123	0.112	0.108	0.099	0.107	0.105	0.105	0.112	0.119
26	imaii	EF216255	0.079	0.092	0.105	0.098	0.109	0.105	0.117	0.110	0.104	0.098	0.113	0.100	0.100	0.116	0.109
27	guanche	AF081354	0.107	0.129	0.111	0.101	0.116	0.103	0.118	0.099	0.113	0.111	0.116	0.113	0.113	0.129	0.128
28	subobscura	M95151	0.076	0.092	0.096	0.085	0.101	0.088	0.098	0.094	0.094	0.094	0.095	0.102	0.102	0.124	0.117
29	madeirensis	AF081355	0.094	0.101	0.097	0.093	0.110	0.092	0.111	0.099	0.103	0.099	0.095	0.105	0.105	0.118	0.111
30	microlabis	EF216258	0.125	0.140	0.127	0.115	0.133	0.119	0.119	0.135	0.125	0.107	0.115	0.118	0.118	0.144	0.148

**Table 2 (continued)** 

	Species	GenBank accession number	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30
1	glabra (No. 001867)	KP257569															
2	glabra (No. 001868)	KP257570															
3	affinis	M95140															
4	algonquin	M95144															
5	azteca	M95146															
6	athabasca	M95141															
7	tolteca	M95152															
8	narragansett	M95149															
9	helvetica	EF216252															
10	lowei	M95142															
11	miranda	M95148															
12	persimilis	M95143															
13	pseudoobscura	M95150															
14	limingi	EF216256															
15	tsukubaensis	EF216261															
16	tristis	EF216262															
17	obscura	AF081356	0.082														
18	ambigua	M95145	0.067	0.050													
19	hubeiensis HB	EF216253	0.095	0.103	0.076												
20	hubeiensis YN	EF216254	0.095	0.103	0.076	0.001											
21	luguensis	EF216257	0.095	0.111	0.084	0.017	0.015										
22	sinobscura	EF216259	0.091	0.105	0.078	0.004	0.003	0.015									
23	subsilvestris	EF216260	0.113	0.114	0.083	0.079	0.078	0.083	0.081								
24	dianensis	EF216251	0.107	0.106	0.079	0.074	0.073	0.084	0.076	0.039							
25	bifasciata	M95147	0.145	0.153	0.114	0.080	0.078	0.085	0.078	0.088	0.082						
26	imaii	EF216255	0.155	0.153	0.112	0.091	0.089	0.085	0.093	0.090	0.094	0.039					
27	guanche	AF081354	0.141	0.159	0.131	0.121	0.119	0.119	0.123	0.121	0.115	0.133	0.127				
28	subobscura	M95151	0.126	0.126	0.102	0.078	0.076	0.080	0.080	0.081	0.076	0.092	0.091	0.085			
29	madeirensis	AF081355	0.146	0.142	0.109	0.098	0.098	0.102	0.102	0.086	0.081	0.098	0.100	0.090	0.040		
30	microlabis	EF216258	0.135	0.149	0.128	0.117	0.115	0.123	0.117	0.131	0.128	0.117	0.130	0.127	0.110	0.127	

al., 2007) in MEGA5 (Tamura et al., 2011), in order to conduct a distance-based species delimitation. We use the same method as Fartyal et al. (2013) for observing external morphological characters, measuring morphometric characters and dissecting organs. The male terminalia and cibarium of the new species were microphotographed and drawn following Li et al. (2014). We follow McAlpine (1981) for morphological terminology, Zhang and Toda (1992) for definitions of measurements and indices. Type specimens are deposited in the Kunming Natural History Museum of Zoology, Kunming Institute of Zoology, Chinese Academy of Sciences (KIZ).

#### 3 Results

#### 3.1 Species delimitation with DNA sequences

The pairwise K2P distances in the *obscura* group are shown in Table 2. The two newly determined sequences exhibit a distance of 0.0153. Though this distance is comparable with or even greater than the distances between some closely related species of the same group (e.g., 0.0089 between *D. algonquin* and *D. athabasca*, 0.0150–0.0165 between *D. luguensis* and *D. hubeiensis*), greater intraspecific distances can be found in the same subgenus (e.g., 0.0341 within *D. montana*; Mirol *et al.*, 2007). Taking this into account for the two male specimens, which were collected from the same locality, it is reasonable to consider them as conspecific. They are genetically well diverged from the other species employed here for sequences comparison (K2P distance  $\geq$  0.0653), and can be morphologically distinguished from the other members of the *obscura* group. The two male specimens then represent a species new to science.

#### 3.2 Taxonomy

## Drosophila glabra sp. nov. (Figs 1–14)

Description (3). Head. Slightly wider than thorax (Fig. 2). Eye red, with thick interfacetal setulae (Figs 1–3). Ocellar triangle and orbital plates glossy, blackish brown (Fig. 2). Pedicel grayish brown, with two or three prominent and a few small setae (Fig. 3). First flagellomere grayish brown. Frons black, with several interfrontal setulae (Fig. 3). Arista with 3 (3) dorsal and 2 (2) ventral branches besides terminal bifurcation (Fig. 3). Face and facial carina dark brown, facial carina narrowly hunched but flat along midline, broader below (Fig. 3). Clypeus black (Figs 3, 10). Gena black (Fig. 1). Palpus grayish yellow, with acute apex, somewhat triangular in lateral view, with 1 prominent apical seta and several ventral ones (Fig. 4). Lacinia dorsal arms longer, but ventral arm shorter than half length of the anterior arm (Fig. 5). Prementum medially with a pair of small setae between longer setae (Fig. 6). Cibarium anterial margin thickened, anterolateral corners protruded; anterial sensilla 4, quadrangularly arranged; medial sensilla 13 or 14 perside, trichoid, fairly straight, arranged in two rows which slightly diverged anteriorly, anteriorly shorter, anteromost one approximate 1/4 as long as posteromost one; posterior sensilla 14–16 perside, trichoid, slightly curved, arranged in two rows which clearly diverged anteriorly, anterior longer, anteromost extend much past the sensilla campaniformia, approximate 5 times as long as posteromost one and longer than posteromost medial sensillum (Figs 7, 10). Labellum with 7 pseudotracheae per side.

Thorax. Scutum and scutellum glossy, black, without any clear longitudinal stripe (Fig. 2); thoracic pleura glossy, blackish brown to black (Fig. 1). Acrostichal setulae in 8 rows (Fig. 2).

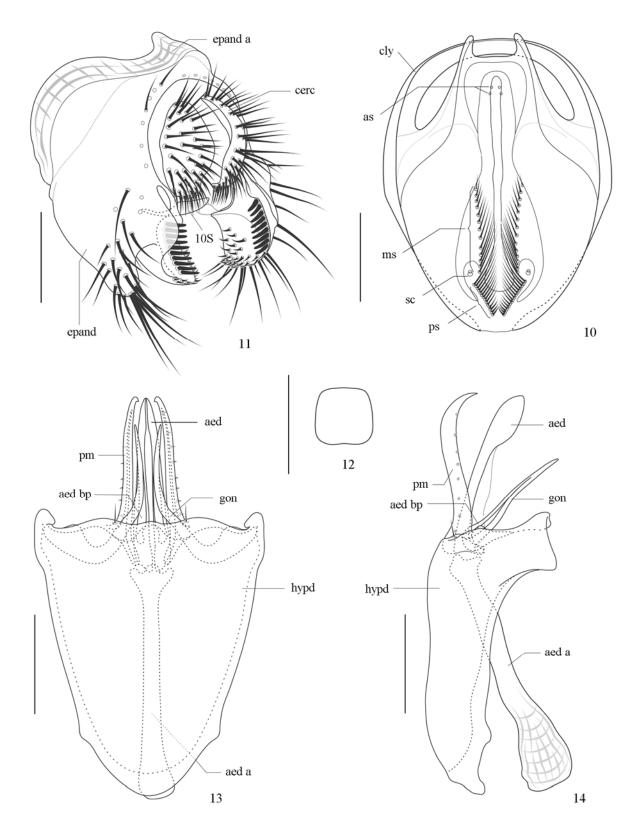
Wing. Hyaline, slightly clouded anteriorly; veins yellowish brown. Halter yellow except for grayish yellow stalk (Fig. 1).

Legs. Coxae and femora blackish brown to black; tibiae blackish brown, darker proximally; tarsi yellowish brown; tibiae and tarsi with recurved setae (Fig. 8). Forelegs. Second tarsomere approximate 3/4 length of first tarsomere; sex-comb teeth 10 or 11 (9 or 10 in paratype) on first tarsomere, 10 (8 or 9) on second tarsomere; both sex-combs arranged slightly oblique against axes of respective tarsomeres (Fig. 9). Preapical setae present on all tibiae; apical seta present on midleg tibia only.

Abdomen. Tergites I and II dark brown, III–(VI+VII) blackish brown to black (Fig. 1); sternites I and II blackish brown, III–V grayish yellow.



Figs 1–9. *Drosophila glabra* **sp. nov.**, holotype, No. 001868. 1. Body, legs and wing, lateral view. 2. Head and thorax, dorsal view. 3. Head, anterior view. 4. Palpus. 5. Lacinia, lateral view. 6. Prementum, ventral view. 7. Cibarium, lateral view. 8. Tibia. 9. Sex-combs on 1st and 2nd foreleg tarsomeres. Scale bars: 1–3=1.0 mm, 4–9=0.1 mm.



Figs 10–14. *Drosophila glabra* **sp. nov.**, holotype, No. 001868. 10. Cibarium. 11. Periphallic organs. 12. Median piece of 10th sternite. 13. Phallic organs, ventral view. 14. Phallic organs, lateral view. Abbreviations: 10S—10th abdominal sternite, aed—aedeagus, aed a—aedeagal apodeme, aed bp—aedeagal basal process, as—anterior sensilla, cerc—cercus, cly—clypeus, epand—epandrium, epand a—epandrial apodeme, gon—gonopod, hypd—hypandrium, ms—medial sensilla, ps—posterior sensilla, pm—paramere, sc—sensilla campaniformia, sur—surstylus. Scale bars=0.10 mm.

Terminalia (Figs 11–14). Epandrium brown, paler on ventral portion, not pubescent, with 7 (6 in paratype) setae per side along posterior margin of upper half and 32 (31) setae on caudao-ventral portion of lower half (Fig. 11). Surstylus brown, with 9 or 10 (8) peg-like prensisetae, 23 (22) trichoid setae, without hook-shaped projection at dorso-caudal corner (Fig. 11). Cercus dark brown, anteriorly connected to epandrium by membranous tissue, somewhat oval, not pubescent, with 44 (38) setae more densely distributed on lower 1/3 (Fig. 11). Median piece of tenth sternite sclerotized, somewhat trapeziform, nearly as long as wide (Fig. 12); lateral piece membranous, broad, slightly sclerotized laterally. Aedeagus brown, narrow (approximate 1/5 as broad as hypandrium in ventral view), articulated with apodeme (Fig. 13); lateral plates sclerotized, apically expanded and basally with triangular knob in lateral view; median membrane hairless (Fig. 14); apodeme 1.44 (1.56) as long as aedeagus, brown, with muscle-attaching portion more or less flat laterally (Figs 13–14). Paramere as long as aedeagus, with approximately 8 minute sensilla arranged longitudinally on subapical to basal portion, apically curved and pointed, falciform in lateral view (Figs 13–14). Gonopod apically fused to aedeagal basal process (Figs 13–14). Hypandrium with a pair of paramedian setae (Figs 13–14).

Measurements (holotype (paratype), in mm). BL (straight distance from anterior edge of pedicel to tip of abdomen) 2.25 (2.50); ThL (medial distance from anterior notal margin to apex of scutellum) 0.92 (0.93); WL (distance from humeral cross vein to wing apex) 2.10 (2.20); WW (maximum wing width) 1.00 (1.05).

Indices (holotype (paratype), in mm). FW/HW (frontal width / head width) 0.49 (0.48); ch/o (maximum width of gena / maximum diameter of eye) 0.18 (0.25); prorb (proclinate orbital seta / posterior reclinate orbital seta in length) 0.86 (1.02); rcorb (anterior reclinate orbital seta / posterior reclinate orbital seta in length) 0.39 (0.41); orbito (distance between proclinate and posterior reclinate orbital seta / distance between inner vertical and posterior reclinate orbital setae) 0.68 (0.76); vb (subvibrissal seta / vibrissa in length) 0.40 (0.39); dcl (anterior dorsocentral seta / posterior dorsocentral seta in length) 0.54 (0.60); sctl (basal scutellar seta / apical scutellar seta in length) 0.93 (0.85); sterno (anterior katepisternal seta posterior katepisternal seta in length) 0.31 (0.50); mid katepisternal seta indistinguishable from the other fine setae; dcp (distance between ipsilateral dorsocentral setae / distance between anterior dorsocentral setae) 0.50 (0.54); sctlp (distance between ipsilateral scutellar setae / distance between apical scutellar setae) 1.21 (1.20); C (2.00 costal section between subcostal break and 2.40 (2.00); 4v (2.00) (2.00); 4v (2.00) (2.00); 4v (2.00) (2.00); 4v (2.00) (2.00); 5v (2.00) (2.00); 5v (2.00) (2.00); 5v (2.00) (2.00); 6v (2.00) (2.00); 6v (2.00) (2.00); 6v (2.00) (2.00); 6v (2.00); 6v (2.00) (2.00); 6v (2.00); 6v (2.00) (2.00); 6v (2.00); 6v (2.00) (2.00); 6v (2.00);

Holotype ♂ (No. 001868), China, Guangxi, Xing'an, Maoershan National Nature Reserve, by banana trap, 19 March 2009, coll. Jian-Jun Gao and Masanori J. Toda (KIZ). Paratype 1♂, (No. 001867), same data as holotype (KIZ).

Distribution. China (Guangxi).

Etymology. The specific name is referring to the hairless median membrane of aedeagus.

Remarks. The new species is distinguishable from other Old World members of the *obscura* group by the combination of the following characters: (1) palpus has a relatively acute apex, giving the entire palpus a somewhat triangular shape in lateral view (Fig. 4); (2) scutum without any clear longitudinal stripe (Fig. 2); (3) foreleg 1st and 2nd tarsomeres with moderately sized sex-combs (with 8–10 teeth), both slightly oblique against the axes of respective tarsomeres (Fig. 9); (4) foreleg 2nd tarsomere approximate 3/4 length of 1st tarsomere (Fig. 9); (5) surstylus without hook-shaped projection at dorso-caudal corner (Fig. 11); (6) median sections of 10th abdominal sternite broad, somewhat trapeziform (Fig. 12); (7) median membrane of aedeagus hairless (not hirsute) (Fig. 14).

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